Translation

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Form PCT/IPEA/416
213/PCT		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP2004/006944	28.06.2004	26.06.2003
International Patent Classification (IPC) or national classification and IPC		
Applicant		
MERCK EPROVA AG		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 7 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☐ *(sent to the applicant and to the International Bureau)* a total of _____ sheets, as follows:
      ☐ sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s))
      _____, containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).
4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the report
☐	Box No. II	Priority
☐	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☐	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
Name and mailing address of the IPEA/EP	Authorized officer
Facsimile No.	Telephone No.

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/EP2004/006944

Box No. I Basis of the report

1. With regard to the language, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ This report is based on translations from the original language into the following language _____, which is the language of a translation furnished for the purposes of:

☐ international search (Rule 12.3 and 23.1(b))

☐ publication of the international application (Rule 12.4)

☐ international preliminary examination (Rule 55.2 and/or 55.3)

2. With regard to the elements of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

☐ the international application as originally filed/furnished

☒ the description:

pages 1-12 as originally filed/furnished

pages* _____ received by this Authority on _____

pages* _____ received by this Authority on _____

☒ the claims:

nos. 1-15 as originally filed/furnished

nos.* _____ as amended (together with any statement) under Article 19

nos.* _____ received by this Authority on _____

nos.* _____ received by this Authority on _____

☐ the drawings:

sheets _____ as originally filed/furnished

sheets* _____ received by this Authority on _____

sheets* _____ received by this Authority on _____

☐ a sequence listing and/or any related table(s) – see Supplemental Box Relating to Sequence Listing.

3. ☐ The amendments have resulted in the cancellation of:

☐ the description, pages _____

☐ the claims, nos. _____

☐ the drawings, sheets/figs _____

☐ the sequence listing *(specify)*: _____

☐ any table(s) related to sequence listing *(specify)*: _____

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

☐ the description, pages _____

☐ the claims, nos. _____

☐ the drawings, sheets/figs _____

☐ the sequence listing *(specify)*: _____

☐ any table(s) related to sequence listing *(specify)*: _____

* *If item 4 applies, some or all of those sheets may be marked "superseded."*

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/EP2004/006944

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1-15	YES
	Claims		NO
Inventive step (IS)	Claims		YES
	Claims	1-15	NO
Industrial applicability (IA)	Claims	1-15	YES
	Claims		NO

2. Citations and explanations (Rule 70.7)

D1: ODIN ELISABETH ET AL: "Chemical stability and human plasma pharmacokinetics of reduced folates" CANCER INVESTIGATION, vol. 16, no. 7, 1998, pages 447-455, XP009041635 ISSN: 0735-7907

D2: WO 97/27764 A (AYLING JUNE E; BAILEY STEVEN W (US); UNIV SOUTH ALABAMA (US)) 7 August 1997 (1997-08-07)

D3: WO 95/26963 A (PHARMACHEMIE BV) 12 October 1995 (1995-10-12)

D4: US-A-4 931 441 (LAWRENCE RICHARD P) 5 June 1990 (1990-06-05)

D5: US-A-5 434 087 (BEGGS MICHAEL J ET AL) 18 July 1995 (1995-07-18)

1. Novelty (PCT Article 33(2))

The subject matter of the present independent claims appears to be novel, since it is not shown in any available prior art document.

D1 (page 451, paragraph entitled "In Vitro Stability of Reduced Folates"; figure 3) discloses

5,10-methylene-THF and a pH of 8.94, but not citrate.

D2 (examples 3-5, 8, 9) discloses citrate in combination with reduced folates, in particular 5-formyl-THF and 5,10-methenyl-THF, but not in combination with 5,10-methylene-THF.

D3, D4 and D5 do not disclose 5,10-methylene-THF.

2. Inventive step (PCT Article 33(3))

2.1. The present claims do not involve an inventive step because they do not solve the technical problem.

The problem addressed by the present invention consists in improving the stability of 5,10-methylene-THF.

According to the description, said problem is solved by adjustment to a basic pH and combination with citrate, thereby producing a pronounced improvement in stability.

(i) In support of this thesis, example 2 provides stability data for lyophilisates of 5,10-methylene-THF and citrate according to the invention at temperatures of -15 and 4 DEG C. These data are compared with results obtained for an untreated reference sample which, however, was investigated at a temperature of 25

DEG C. Since chemical stability and temperature are known to be significantly related, the data are not comparable owing to the different temperatures at which they were obtained and the comparison is therefore not meaningful.

(ii) The same objection applies to the stability data disclosed in example 3, which were all determined in solutions at a temperature of 25 DEG C. Reference is made to D1 (page 451, paragraph entitled "In Vitro Stability of Reduced Folates"; figure 3) for the required comparative sample. However, D1 provides no apparent indication of temperature and it is therefore unclear whether the data from D1 in fact relate to a temperature of 25 DEG C and are therefore comparable. Further, example 3 in the application does not indicate the concentrations of the solutions according to the invention, which are characterized only as "dilute" or "concentrated". As D1 shows, the concentration of 5,10-methylene-THF is crucial to the stability of the substance. Finally, the said data give no information concerning the interaction of citrate and alkaline pH, since example 3 of the application does not show the pH of the solutions investigated.

(iii) Only example 8 shows the concentrations and pH of the solution investigated. The numerical values listed in the table with reference to D1 evidently correspond to the curves designated + (100 - 58 - 38 - 18 - 8) and o (100 - 84 - 12 - 8 - 6) in D1. These curves relate to the

stability of 1 mM 5,10-methylene-THF in the absence (+) or the presence (o) of air. The concentration of about 46.5 mM stated in example 8 for the solution investigated is appreciably higher than that of the solution described in D1 (1 mM) and the findings are therefore not comparable. To summarize: it has been established that, owing to lack of comparability, the experimental data supplied with the present application are not suited to demonstrating that combination with citrate has a positive effect on the stability of 5,10-methylene-THF. In the absence of corresponding data, which should permit direct comparison at identical concentrations and temperatures, the problem of interest cannot be recognized as having been solved and therefore inventive step cannot be recognized as having been established.

2.2. The subject matter of the present claims is suggested by D1 in combination with D3-D5.

D1 (page 451, paragraph entitled "In Vitro Stability of Reduced Folates"; figure 3), which represents the closest prior art, discloses a recovery rate of 25% for 1 mM 5,10-methylene-THF after 20 hours at pH 8.94 compared with 0% at pH 6.6 and refers to the importance of an alkaline pH in relation to the stability of 5,10-methylene-THF. D1 does not suggest a stability-enhancing effect exerted by citrate.

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

On the assumption that the claimed stability-enhancing effect of citrate can in fact be shown, the problem addressed by the present invention consisted in providing a 5,10-methylene-THF formulation having increased chemical stability.

A person skilled in the art knows from the prior art that, in numerous reduced folic acid derivatives, for example, folinic acid (D3: page 1, line 34 to page 2, line 10), 5-formyl-THF (D4: column 2, lines 31-47) and 5-methyl-THF (D5: column 8, lines 26-35), citrate improves stability. Therefore, it would seem obvious to a person skilled in the art to solve the above-mentioned problem by making use of citrate, thus arriving at the subject matter of the present claims.

That the salt form exerts a positive effect on the stability of reduced folates, including 5,10-methylene-THF, is known from D2 (page 17, lines 8-10).

With respect to all further features of the present claims, no special effect which could establish inventive step is presently discernible.